

ACUTE COAGULOPATHY OF TRAUMA IN THE RAT

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ABSTRACT—Introduction: Acute coagulopathy of trauma (aCOT) is a state of disordered coagulation developing soon after severe injury and blood loss and has been defined in the clinical literature as an elevation in prothrombin time (PT) and activated partial thromboplastin time (aPTT). **Objective:** The purpose of this study was to develop a rat model of aCOT resulting from polytrauma and hemorrhage and showing an elevation in PT and aPTT. **Methods:** Sprague-Dawley rats (300–400 g) were anesthetized with isoflurane. Polytrauma was induced by damaging 10 cm of small intestines, the right and medial liver lobes, the right leg skeletal muscle, and fracture of the right femur. Rats were hemorrhaged 40% of their estimated blood volume. No resuscitation was given. Venous and arterial blood samples were taken at times up to 4 h. **Results:** Polytrauma and hemorrhage resulted in a significant rise in PT, aPTT, potassium, lactate, and glucose. There was a significant decrease in plasma bicarbonate, base excess, and sodium. Blood urea nitrogen and creatinine rose steadily throughout the 4 h indicative of progressive renal failure. Hematocrit decreased significantly immediately after hemorrhage and trauma indicating a movement of fluid into the vascular space from extravascular sources, which was mirrored by a decrease in plasma fibrinogen concentration. In contrast, platelet count initially decreased, rose at 2 h, and decreased again at 3 to 4 h, indicating that platelets were released into the vascular space. The change in platelet count was mirrored by the changes in thrombin-antithrombin and plasmin-antiplasmin complexes. Rotational thromboelastometry showed complex changes. Clotting firmness fell initially, rose at 2 h, and fell again at 3 to 4 h similar to the changes in platelet count. α Angle was elevated, and clotting time was shortened over the 4 h. Treatment with cytochalasin D (platelet function inhibitor) eliminated the increases in clotting firmness and thrombin generation seen at 2 h with rising platelet count. **Conclusions:** This model of aCOT in rats showed complex changes in clotting parameters over 4 h that included a rise in PT and aPTT. At 4 h, there was a decrease in clotting firmness, even though the clot formation was faster (elevated α angle and decrease in clotting time). The decrease in clotting firmness correlated with falling fibrinogen and platelet count. This model affords an opportunity to evaluate interventions in the treatment of aCOT.

KEYWORDS—Coagulation, polytrauma, hemorrhage, clotting firmness, platelets, fibrinogen, PT, aPTT

INTRODUCTION

Acute coagulopathy of trauma (aCOT) is a hypocoagulable state that develops soon after severe injury and blood loss. It results in continuous bleeding, irrespective of surgical intervention, and is of extreme concern as trauma patients with coagulopathy have a higher mortality rate than similarly injured patients who are not coagulopathic (1–6). Trauma associated with coagulopathy results in an increase in transfusions, organ dysfunction, and critical care unit days (2, 7) and occurs in 25% and 38% of the severely injured civilian and military trauma populations, respectively (1, 8). Platelet dysfunction has been reported in trauma patients (9), and a small percentage have a hyperfibrinolysis (10). The concept of hemostatic resuscitation with a balanced plasma-to-red blood cell ratio

as part of a Damage Control Resuscitation paradigm was developed to specifically decrease the rate of symptomatic coagulopathy in these severely injured patients (11, 12). Other resuscitation regimens have been proposed to deal with the deficit in coagulation and include prothrombin complex concentrate, recombinant activated FVII, tranexamic acid, and fibrinogen (13, 14). The degree of coagulopathy that develops in trauma patients has been correlated with the amount of tissue damage (15, 16) and a fall in vascular perfusion due to hemorrhage (7, 17, 18).

A prolongation in prothrombin time (PT) and activated partial thromboplastin time (aPTT) in humans and experimental animals that develops soon after trauma is the hallmark of aCOT (16, 19, 20). The cause of the coagulopathy is probably multifactorial and may include systemic and massive activation of coagulation and anticoagulant systems and fibrinolysis (21). Recent evidence shows a dysfunction in multiple areas including platelet function (9, 22), regulation of fibrinolysis (10, 17, 23), and an elevation in activated protein C (7, 24). Any or all of these dysfunctions could result in disseminated intravascular coagulation, weak clot formation, premature clot lyses, and inhibition of thrombin generation.

The purpose of this study was to develop an experimental model of aCOT in the rat that included polytrauma and hemorrhage and demonstrated an elevation in PT. This model

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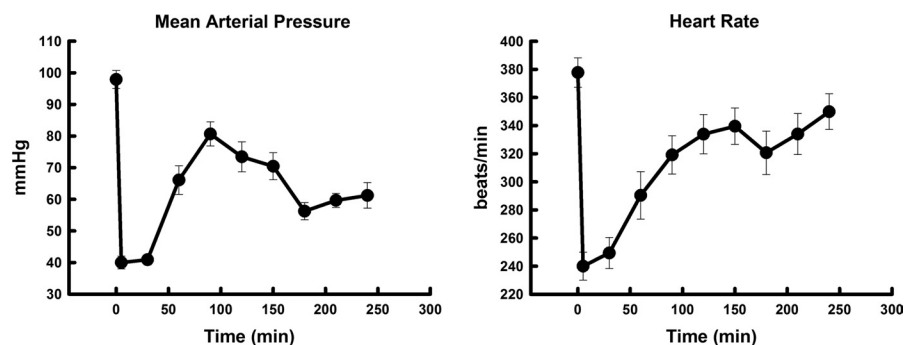


FIG. 1. Mean arterial blood pressure and heart rate measured in nine rats after trauma and 40% hemorrhage. 0 Time taken before trauma and hemorrhage. Values represent mean \pm SE.

would allow for the systematic study of aCOT and identify the specific mechanisms underlying the deficits in coagulation that occur after severe trauma and hemorrhage.

METHODS

This study was approved by the Animal Care and Use Committee of the US Army Institute of Surgical Research. All animals received care and were used in strict compliance with the Guide for the Care and Use of Laboratory Animals.

Sprague-Dawley rats (300–400 g) were anesthetized with isoflurane/100% oxygen through a nosecone and allowed to breathe spontaneously. Cannulas were placed in the left femoral artery and vein for measurement of arterial blood pressure and to obtain vascular access. Polytrauma was induced by crush injury to the small intestines, the left and medial liver lobes, and the right leg skeletal muscle and by fracture of the right femur. A midline incision was made through the abdominal skin and underlying muscle layers. A 10-cm section of small intestines was isolated just anterior to the cecum and run gently through a clamp covered with silastic tubing with a 2-mm distance between the clamps to cause a gentle crush injury. The right and medial lobes of the liver received three crushes to each lobe using the same clamp. The intestines and liver were replaced, and the abdominal incision closed with sutures in two layers. To fracture the left femur, we use a modification of a 3-point impact device (25) by dropping six stainless-steel balls (65 g each) stacked together, from 36" through a guide tube (1" internal diameter) to impact on a rounded aluminum blade resting on the mid-right femur with the right leg resting on two aluminum stands, one under the hip and one under the knee. A large hemostat (5 in tongs) was used to clamp the muscle of the right leg 10 times. The rats were then bled to lower mean arterial pressure to 40 mmHg within 5 min and maintained at 40 mmHg until 40% of estimated blood volume was removed. Hemorrhage was usually completed between 30 and 60 min. Hemorrhage was then discontinued, and blood pressure and heart rate were allowed to freely compensate (Fig. 1). No resuscitation was

given. Blood volume was estimated as 7% of body weight (26–28). The polytrauma was performed within a 5- to 10-min window followed immediately by hemorrhage. The order of trauma was intestinal damage first, followed by liver and femur fracture, then leg skeletal muscle damage. The crush injury was intended to cause microvascular damage, as opposed to cutting, chopping, or mincing of the tissue, which would have led to uncontrolled hemorrhage.

Different groups of rats were killed (exsanguination via the femoral vein or artery under isoflurane) at 30, 60, 120, 180, and 240 min after the beginning of hemorrhage. Rats killed at time 0 (baseline) had no trauma or hemorrhage, but had their left femoral artery and vein cannulated. Each time point represented a group of rats with $n = 7$ –11/group for all the variables measured. Blood samples (1 mL) were drawn over 100 μ L of 200 mM sodium citrate, 23.5 IU heparin, or 100 μ L hirudin (25 μ g/mL) as anticoagulants, as appropriate for different assays.

Blood chemistry was measured from 1 mL of arterial blood (lithium heparin) by ISTAT (Abbott Laboratories, Abbott Park, Ill) in single determinations. Prothrombin time, aPTT (venous plasma), and fibrinogen concentration (arterial plasma) were measured on a STA-R Evolution (Diagnostica Stago, Parsippany, NJ) from citrated blood in duplicates. Thrombin antithrombin (TAT) and plasmin antiplasmin (PAP) were measured in citrated arterial plasma in duplicates by enzyme-linked immunosorbent assay (ELISA) (Life Sciences Advanced Technologies Inc, St Petersburg, Fla). Soluble fibrin monomer was measured by ELISA (MyBioSource.com, San Diego Calif) in arterial plasma from citrated blood in duplicates. Rotational thromboelastometry was performed in citrated arterial blood on a ROTEM Delta (TEM, Munich, Germany). To obtain a functional profile of coagulation, the Extem assay (in quadruplicates) was used to evaluate the extrinsic pathway using tissue factor to initiate coagulation as would be expected following tissue injury. Cytochalasin D (inhibit platelet function in the Fitem assay, in duplicates) and aprotinin (inhibit fibrinolysis in the Aptem assay, in duplicates) were used to discern coagulation components associated with the coagulopathy observed. Platelet count was measured in hirudin-treated arterial blood on an ABX Pentra 120 hematology analyzer (ABX Diagnostics, Inc, Irvine, Calif) in duplicate. All assays and tests were run by the manufacturer's specifications (Table 1).

TABLE 1. Interpretation of clotting parameters

Parameter	Interpretation
PT	Time to clot formation via the extrinsic and common pathway
aPTT	Time to clot formation via the intrinsic and common pathway
Fibrinogen	Substrate for thrombin to produce a fibrin clot
Fibrin monomer	Intermediate between fibrinogen and fibrin clot
TAT	Formation of TAT complex and indicative of thrombin generation
PAP	Formation of PAP complex and indicative of plasmin generation
Mean clotting firmness	Clot strength
α Angle	Speed of clot formation
Clotting time	Time to detection of the initial clot formation by ROTEM

Data analysis

One-way analysis of variance (ANOVA) was used to determine significance between time points when population distribution was normal (Kolmogorov-Smirnov test). Holm-Sidak post hoc test was used to compare to time 0. Kruskal-Wallis one-way ANOVA on ranks was used for non-normally distributed data. Dunn post hoc test was used to compare to time 0. Correlation was performed using a linear model on platelet count and fibrinogen concentration versus mean clotting firmness (MCF). Statistics were performed by SigmaPlot (Systat Software, Inc, San Jose, Calif.). Significance was set at $P < 0.05$.

RESULTS

Polytrauma and hemorrhage

Upon postmortem inspection, we found that most of the rats had only small amounts of petechial hemorrhaging in the traumatized intestines. The liver lobes had three lines of hematoma each in the medial and right lobes, but no overt bleeding into the abdominal cavity. The femur usually showed a break close to the knee, sometimes with obvious knee injury. There was a small hematoma at the site of the femur break

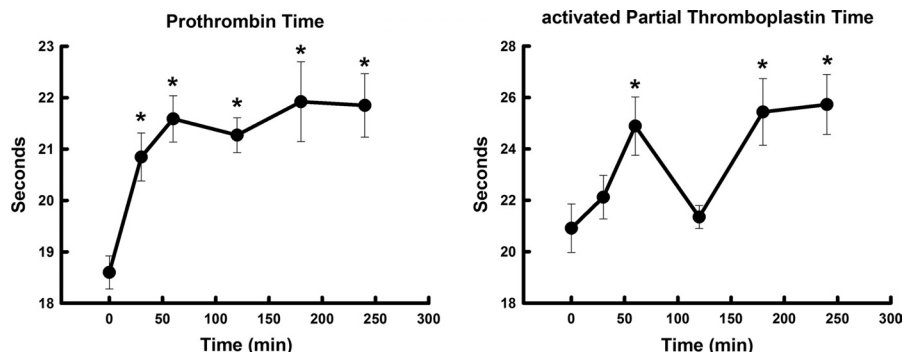


FIG. 2. Prothrombin time and aPTT measured in venous plasma taken from groups of rats killed at each time point ($n = 7-9/\text{group}$). Values represent mean \pm SE. * $P < 0.05$ compared with time 0 after ANOVA.

(approximately 0.25 mL). The damaged skeletal muscle showed no bleeding, but was pale compared with adjacent abdominal skeletal muscle. It appeared that very little uncontrolled hemorrhage had occurred in this model. The cardiovascular changes after trauma and hemorrhage are shown in Figure 1. Heart rate fell with blood pressure after hemorrhage and began to recover as blood pressure rose. Blood pressure then peaked at about 80 mmHg and slowly fell to about 55 mmHg at 3 h and leveled off. Heart rate continued to recover over the 4-h experimental period, but never returned to baseline levels.

PT and aPTT

Polytrauma and hemorrhage resulted in a significant prolongation in both PT and aPTT in this model, recapitulating the clinically described coagulopathy (Fig. 2). Prothrombin time and aPTT were significantly elevated above control over the 4 h, except for aPTT at 2 h. Prothrombin time and aPTT were measured in venous plasma.

Blood chemistry

There was a significant fall in bicarbonate and base excess and rise in plasma lactate and glucose after trauma and hemorrhage (Fig. 3). However, an acidosis was not evident by pH, which rose significantly by 4 h, most likely due to hyperventilation because arterial PCO_2 fell significantly (Fig. 4). Trauma and hemorrhage also demonstrated a fall in plasma sodium and rise in potassium (Fig. 4). Blood urea nitrogen and creatinine rose steadily throughout the 4 h, suggesting the onset of acute renal failure (Fig. 5). Hematocrit and hemoglobin fell significantly immediately after trauma and hemorrhage, indicating a movement of fluid into the vascular space from extravascular sources (Fig. 5).

Coagulation parameters

Fibrinogen concentration fell significantly immediately after trauma and hemorrhage and remained low during the entire experiment (Fig. 6). Fibrin monomer also decreased over time, but more slowly than fibrinogen. Platelet count changed significantly after trauma and hemorrhage (Fig. 7). Initially, platelet count fell, then rose at 2 h to near baseline, only to fall again at 3 and 4 h. This was mirrored by the changes in TAT and PAP (Fig. 8), which are reflective of the generation of thrombin and plasmin, respectively.

ROTEM

Polytrauma and hemorrhage led to a complex change in MCF, α angle, and clotting time (Figs. 9–11). Mean clotting firmness initially decreased, then rose at 2 h and decreased again by 4 h, mirroring the change in platelet count (Extem). Inhibiting platelet function with cytochalasin D completely abolished the rise at 2 h and significantly decreased the baseline MCF to approximately 20% (Fibtem). Inhibition of fibrinolysis with aprotinin had no significant effect on MCF (Aptem). α Angle rose significantly immediately after trauma and hemorrhage and remained elevated (Fig. 10). The rise in α angle was eliminated after inhibition of platelet function. Inhibition of fibrinolysis did not affect the rise in α angle. Clotting time fell significantly at 60 min and remained low throughout the experiment (Fig. 11).

DISCUSSION

We have successfully developed a polytrauma/hemorrhage model in the rat that recapitulates clinical descriptions of an aCOT as evidenced by a prolongation in PT and aPTT. The

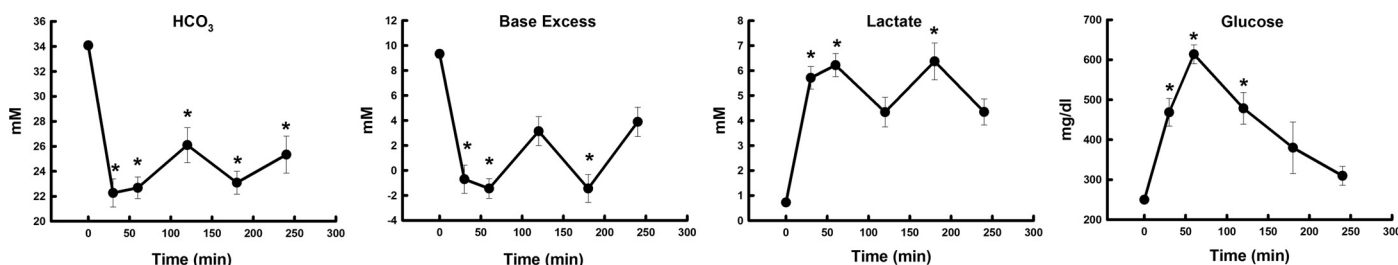


FIG. 3. Bicarbonate (HCO_3^-), base excess, lactate, and glucose measured in arterial blood taken from groups of rats killed at each time point ($n = 7-10/\text{group}$). Values represent mean \pm SE. * $P < 0.05$ compared with time 0 after ANOVA.

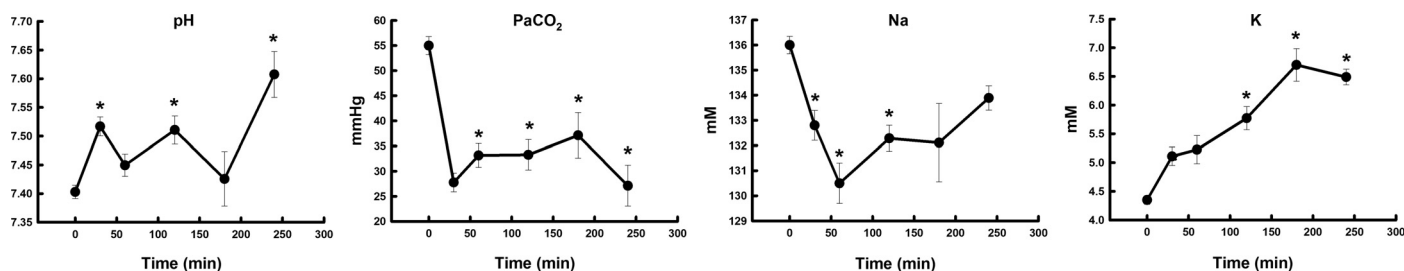


FIG. 4. pH, PaCO₂, sodium, and potassium measured in arterial blood taken from groups of rats killed at each time point ($n = 7-10/\text{group}$). Values represent mean \pm SE. * $P < 0.05$ compared with time 0 after ANOVA.

severity of the trauma/hemorrhage was further evidenced by the rise in plasma lactate, glucose, and potassium and fall in bicarbonate and sodium. The rise in blood urea nitrogen and creatinine reflected a progressive deterioration in renal function. Clotting firmness as measured by ROTEM (Fig. 9) demonstrated a series of changes that tracked the changes in platelet count (Fig. 7). Mean clotting firmness initially fell, then rose, and then fell again. These changes were completely eliminated after inhibition of platelet aggregation with cytochalasin D. α Angle was elevated, and clotting time was shortened, suggesting that coagulation factors were activated and adequate to support thrombin production. Again, these changes were eliminated after treatment with cytochalasin D. This suggests that about 80% of the clotting firmness was due to platelets. It also suggests that platelets play an important role in the speed of clot formation (rise in α angle). Mean clotting firmness was reduced by 4 h in conjunction with the fall in fibrinogen and platelet count (Figs. 6 and 7). Thus, by ROTEM analysis, it seems that the clot formed faster; however, the clot strength was weaker (4 h). Furthermore, without platelets, both the overall strength of the clot and the ability to generate thrombin are greatly reduced.

In this model, we found that fibrinolysis was a negligible component in the development of aCOT according to ROTEM with aprotinin (Figs. 9 and 10). The changes in MCF and α angle were not different with or without inhibition of fibrinolysis. Previous studies have suggested that the coagulopathy seen after trauma may be due to a hyperfibrinolytic state resulting in accelerated clot breakdown and a weaker clot (10, 17, 23). Fibrinolysis had little effect on our model and may not be an important component of the coagulopathy observed in our study.

The precipitous fall in hematocrit has been described previously in rats after hemorrhage (26–28) and demonstrates how rapidly fluid moves into the vascular space from extravascular sources, irrespective of external resuscitation. This

cell-free fluid dilutes the remaining red blood cells and has been used to estimate restoration of blood volume (26–28). This restitution probably accounts for some of the recovery of arterial pressure (Fig. 1). Because platelet count did not fall to the same degree as hematocrit, platelets either must be released from storage (spleen or bone marrow) or are newly formed from megakaryocytes. The rise in platelets at 2 h supports this proposition. However, by 4 h, platelet count was down and probably reflects dilution and consumption. These observations warrant further investigations.

Acute coagulopathy has been described in patients with severe trauma and is correlated with the degree of injury (1). Furthermore, patient survival is directly correlated to the degree of coagulopathy and the elevation in PT and aPTT (2, 4, 17, 20), making aCOT a serious clinical issue that must be addressed. Interestingly, in order for coagulopathy to develop, it appears that the injury must also contain an element of blood loss or hypoperfusion of vascular beds, as coagulopathy does not manifest from hemorrhage or trauma alone (20, 24).

Fibrinogen and platelets are essential for clot formation. Fibrinogen therapy has been used successfully in trauma patients to bring elevated PT and aPTT back to normal (29). Fibrinogen availability to support hemostasis is a function of baseline fibrinogen concentration in plasma, synthesis, and secretion from the liver and metabolism or consumption (30). In swine subjected to hemorrhage and resuscitation, fibrinogen concentration has been shown to be elevated after 24 h (31, 32) and is probably due to an elevation in synthesis of acute-phase response proteins (31). In the present study, hemorrhage and trauma led to a precipitous fall in fibrinogen concentration that mirrored the fall in hematocrit. Because no resuscitation fluid was given in these experiments, dilution occurred because of normal autorestitution of vascular volume and contributed substantially to the low fibrinogen concentration. However, with the degree of trauma sustained in this model, consumption of fibrinogen must be considered to be additive to dilution.

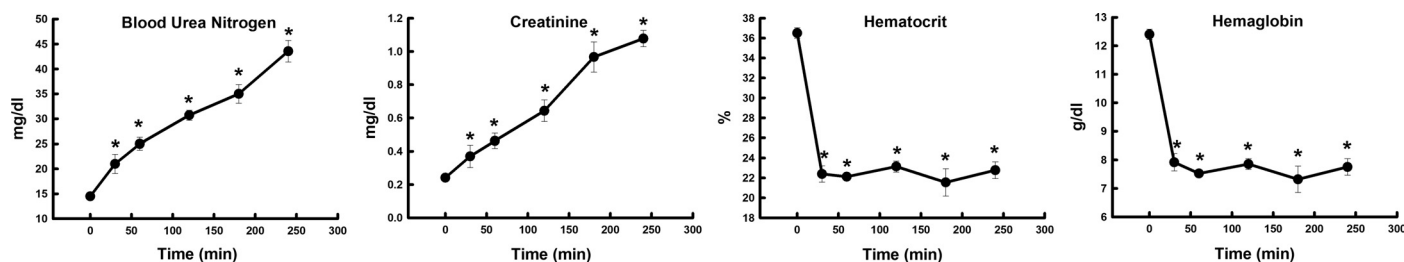


FIG. 5. Blood urea nitrogen, creatinine, hematocrit, and hemoglobin measured in arterial blood, taken from groups of rats killed at each time point ($n = 7-10/\text{group}$). Values represent mean \pm SE. * $P < 0.05$ compared with time 0 after ANOVA.

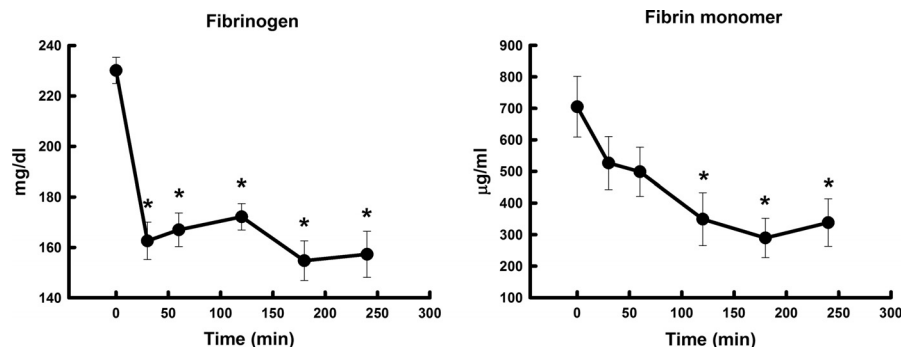


FIG. 6. Fibrinogen concentration and soluble fibrin monomer measured in arterial plasma taken from groups of rats killed at each time point ($n = 6-9/\text{group}$). Values represent mean \pm SE. * $P < 0.05$ compared with time 0 after ANOVA.

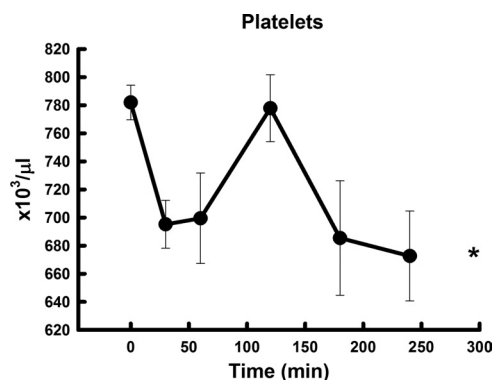


FIG. 7. Platelet count measured in arterial blood taken from groups of rats killed at each time point ($n > 9/\text{group}$). Values represent mean \pm SE. *Significant response by ANOVA.

This is supported by the high concentration of fibrin monomer observed immediately after trauma, which fell more slowly than fibrinogen.

The changes in plasma fibrinogen concentration in this study were different than the changes in platelet count, suggesting that stored or newly formed platelets are entering the circulation. Although both fibrinogen and platelets play a major role in clot formation, it is interesting to note that the changes in MCF mirrored the changes in platelet count. Correlating the changes in MCF with the changes in fibrinogen or platelets, we found that fibrinogen concentration showed a poorer correlation ($r^2 = 0.49$) than did the changes in platelet count ($r^2 = 0.73$). Changes in TAT and PAP also mirrored

changes in platelet count and most likely reflect the generation of thrombin and plasmin as the platelets provide a surface for conversion of the proenzymes.

This model of polytrauma shows a complex array of coagulation changes that occurs over time that involves significant activation of both the procoagulant and anticoagulant pathways. ROTEM describes the functional properties of the clot to be first hypercoagulable: elevated α angle, shortened clotting time, and rise in firmness at 2 h. The 2-h rise in platelet count had a significant effect on the increase in clotting firmness. However, the elevation in PT and aPTT and fall in fibrinogen and clotting firmness (4 h by ROTEM) indicate an evolution to a hypocoagulable state. This contradiction is also reflected in the clinical literature (21, 33, 34). Severe trauma patients show an elevation in PT, aPTT, fibrin, and fibrinogen degradation products, D-dimers, and a fall in fibrinogen activity (21, 23, 35, 36). The model described here suggests that coagulopathy evolves over time and includes both states (hyper and hypo) in a changing temporal pattern. Our model also suggests a large dilutional component to this coagulopathy as hematocrit was significantly diluted, even though no resuscitation fluid was given. This model did not detect a hyperfibrinolytic state.

Our polytrauma and hemorrhage rat model characterized the early events in the development of coagulopathy over the 4-h period after injury and hemorrhage and may lead to insights into the cause of aCOT in human trauma patients. The strength of this model lies in our ability to study aCOT without complications due to interventions, such as resuscitation, which are normally present in studies using trauma patients and can

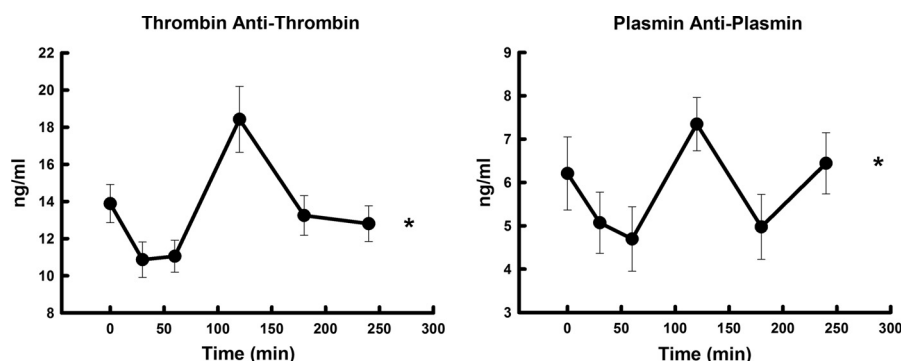


FIG. 8. Thrombin antithrombin and PAP as measured by ELISA from arterial plasma taken from groups of rats killed at each time point ($n = 7-10/\text{group}$). Values represent mean \pm SE. *Significant response by ANOVA.

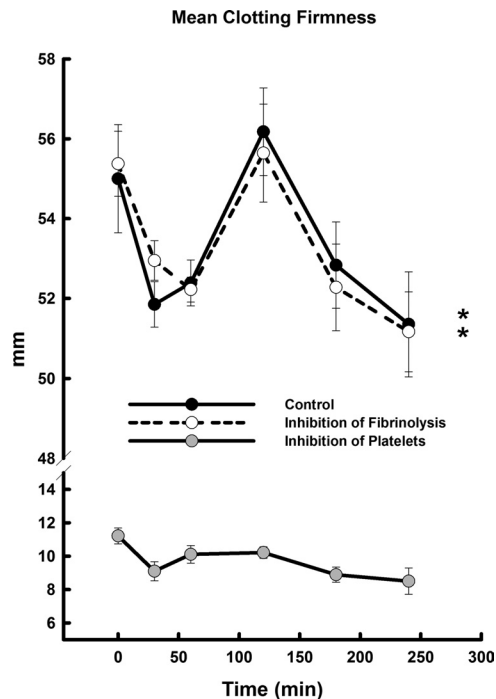


FIG. 9. Mean clotting firmness as measured by rotational thromboelastography from arterial blood taken from groups of rats killed at each time point ($n = 7-11/\text{group}$). Clot initiation by tissue factor alone (Extem control, closed circle) or with the addition of aprotinin to inhibit fibrinolysis (Aptem, open circle), or of cytochalasin D to inhibit platelet function (Fibtem, gray circle). Values represent mean \pm SE. *Significant response by ANOVA.

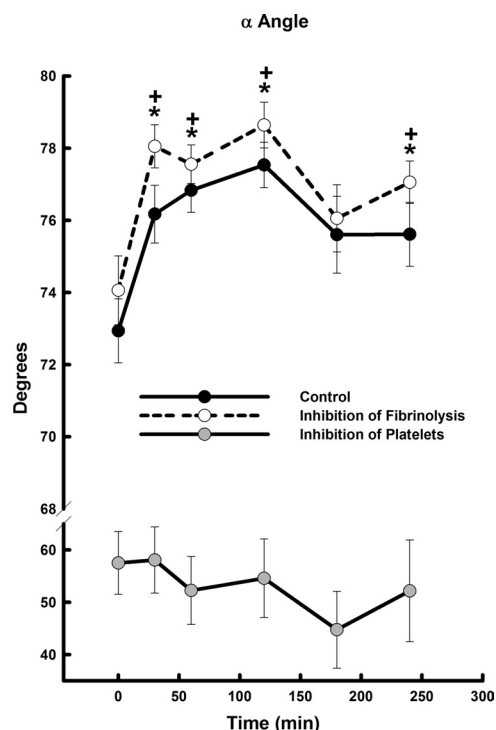


FIG. 10. α Angle measured by rotational thromboelastography from arterial blood taken from groups of rats killed at each time point ($n = 7-11/\text{group}$). Clot initiation by tissue factor alone (Extem control, closed circle) or with the addition of aprotinin to inhibit fibrinolysis (Aptem, open circle), or of cytochalasin D to inhibit platelet function (Fibtem, gray circle). Values represent mean \pm SE. * $P < 0.05$ compared with time 0 after ANOVA.

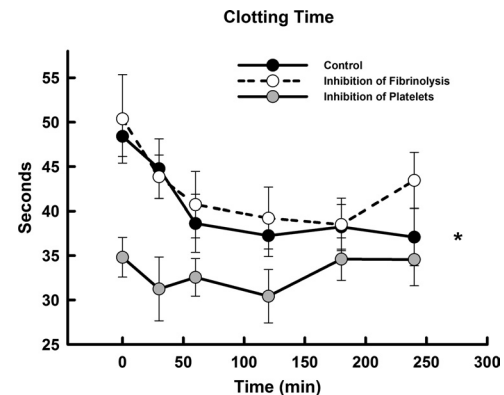


FIG. 11. Clotting time (CT) as measured by rotational thromboelastography from arterial blood taken from groups of rats killed at each time point ($n = 7-11/\text{group}$). Clot initiation by tissue factor alone (Extem control, closed circle) or with the addition of aprotinin to inhibit fibrinolysis (Aptem, open circle), or of cytochalasin D to inhibit platelet function (Fibtem, gray circle). Values represent mean \pm SE. *Significant response by ANOVA.

affect the coagulation profile being studied. Irrespective of these limitations, retrospective evaluations have shown that trauma patients have a significantly elevated PT and aPTT (3, 37) and a fall in fibrinogen concentration (3, 37, 38) as compared with controls. Our model showed similar responses. There is also evidence that trauma patients have significantly elevated α angle and decreased R-time (thromboelastography) as compared with normal controls (37, 39, 40), which is similar to the thromboelastography results of our rat study. Clinical trials measuring clot strength are conflicting and include reports of increases (37, 39) and decreases (40) in clotting strength as compared with control patients and may reflect the results of interventions before measurement or the amount of time that passed between injury and measurement. However, it must be pointed out that our rat model also demonstrated complex changes in clot strength that included a fall, rise, and fall that varied with platelet count.

Limitations

This preparation was not intended to exactly model combat or civilian casualties as we did not include resuscitation, mechanical ventilation, analgesics, or any other prehospital care that would most certainly be performed in a timely manner. The purpose of this preparation was to develop a rat model of aCOT that was free of dilutional coagulopathy (resuscitation) and study the natural time course for the development of the coagulopathy. The purpose of the polytrauma was to cause microvascular damage to a number of different organs, without disrupting major vessels and causing uncontrolled bleeding. This trauma, combined with a defined and controlled hemorrhage, was designed to cause reproducible changes in physiologic parameters that approximate clinical findings in major trauma. We felt that controlling the hemorrhage (40% of blood volume) would lead to more reproducible results. To this end, we realize that this preparation is not exactly like what would be encountered in real critical care situations. However, studying the natural history of the coagulopathy in this model may lead to the development of better resuscitation therapies for the treatment of aCOT in the future.

SUMMARY

We have created a rat model of coagulopathy using poly-trauma plus hemorrhage. This model shows a rise in both PT and aPTT that is similar to what has been described clinically in patients with severe injury and aCOT. This model of aCOT shows a weaker clot at 4 h, even though clot initiation is faster. The weak clot is likely due to a fall in fibrinogen concentration and in platelet count. This rat model mimics what is seen in severe trauma patients and can be used to develop novel resuscitation strategies that can attenuate or eliminate the deficits in coagulation parameters and thereby improve survival.

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